the NMR observables and compared to the published structures. With respect to the explicit membrane, the disposition of Pf1 TM helix was identified and the dynamic nature of the periplasmic helix orientation was observed.

1134-Pos Board B26
Biophysical Characterization and Conformational Analysis of the GTPase Domain of Pre-Protein Import Receptors atToc33G and psToc34G
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atToc33 and psToc34 are GTPases-, that act as receptors for pre-protein import in the chloroplast outer envelope. Various lines of evidence suggest that the GTPase domain of atToc33 (atToc33G) dimerizes, including native PAGE studies (Weibel et al., 2003), gel-filtration experiments (Yeh et al., 2007) and filter binding assays (Koenig et al., 2008). The crystal structure of atToc33 shows that it is a monomer (Koenig et al., 2008) while psToc34 crystallizes as a dimer (Sun et al., 2002). The R130A atToc33 mutant apparently leads to monomer formation *in vitro* (Reddick et al., 2007; Weibel et al., 2003). Both-, atToc33-wt and atToc33-R130A bind GTP and GDP with high affinity and hydrolyse GTP with similar efficiencies (Weibel et al., 2003). To further investigate the conformations of these GTPases, and to characterize their optical spectroscopic properties, we first, expressed atToc33G and its R130A mutant in high yield. Initial characterization of these proteins by CD spectroscopy revealed significant α-helical content in their secondary structure. We also examined the effect of an increase in protein concentration of atToc33G and its R130A mutant using CD and fluorescence spectroscopy. CD spectral line shape does not change with increasing protein concentrations indicating that no or only small conformational changes occurs as the concentration increases. Fluorescence spectra for both proteins are superimposable with an intensity maximum at ~320nm. The addition of GDP to both the proteins leads to reduction of intensity. No wavelength shift in emission spectrum was observed. Our spectroscopic results and anisotropy experiments show that both atToc33G and atToc33G R130A are monomeric, irrespective of the protein concentration and also in presence of GDP or GTP. Experiments to compare atToc33G and atToc33G R130A with their corresponding homologues from *Pisumsativum* are also presented.

1135-Pos Board B27
Binding and Oligomerization of Bcl-2 Family Proteins on Supported Lipid Bilayers with Single Molecule Resolution
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Bid, Bax and Bcl-XL are apoptosis-regulating proteins that play a key role in mitochondrial outer membrane permeabilization during programmed cell death. Elucidating the molecular mechanisms regulating the function of these proteins is essential to understanding how apoptosis can be controlled with drugs, i.e. increased in diseases such as cancer, or decreased in the case of stroke. Cleaved Bid (cBid) recruits Bax to the mitochondrial membrane, which forms oligomeric pores and triggers cell death through the release of cytochrome c into the cytoplasm. Bcl-XL is also recruited to the membrane by cBid, but inhibits apoptosis. In this study, fluorescent confocal microscopy is used to characterize the membrane-binding behaviour of cBid to a mitochondria-like supported lipid bilayer in the presence and absence Bax and Bcl-XL. By working at dilute concentrations of fluorescent protein, single molecular complexes could be detected by fitting Gaussian profiles to diffraction-limited spots. The oligomerization state of cBid was determined by normalizing the fluorescence intensity by the brightness of a cBid monomer, as measured using fluorescence fluctuation techniques. This method revealed two main populations of cBid molecules: monomers which diffuse in the plane of the membrane and higher order oligomers which are predominantly immobile. This suggests that Bid goes from a membrane associated to a membrane inserted conformation due to homo-oligomerization.

1136-Pos Board B28
Single-Molecule Observation of Folding and Insertion of Outer Membrane Proteins in Droplet Interface Bilayers
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Outer membrane proteins (OMPs) are key players in the interface between Gram-negative bacteria and the environment. OmpA serves as an archetypical model of β-barrel membrane protein folding and insertion [1]. Here we use TIRF (Total Internal Reflection Fluorescence) and FRET (Förster Resonance Energy Transfer) microscopy in a droplet interface bilayer system [2] to investigate the folding and insertion on a single-molecule level.
[1] Kleinschmidt, J.H. *Cellular and Molecular Life Sciences* 2003 **60**, 1547
[2] Thompson, J. R. *et al*. *Nano Letters* 2007 **7**, 3875

1137-Pos Board B29
Single Molecule Studies of the General Secretory System
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In bacteria and archaea many proteins use the protein conducting channel SecYEG either to transport across the membrane bilayer or to integrate into the bilayer. Further, it is known that the ATPase SecA binds SecYEG to perform translocation. Crystal structures of detergent-solubilized SecYEG and SecA bound to SecYEG have been reported [Nature 427, 36 (2004); Nature 455, 936 (2008)]. In recent years atomic force microscopy (AFM) has emerged as an important complementary tool to study membrane proteins at the single molecule level in near native conditions. In this work we study two central components of the bacterial secretory system (SecYEG and SecA) in membrane via AFM. We have obtained images of proteoliposomes containing just SecYEG, and SecYEG proteoliposomes assembled in the presence of SecA (SecY·A). All samples were adsorbed on mica surfaces and imaged in aqueous buffer solution. We collected several hundred images of each sample to provide statistics. Heights of SecYEG and SecY·A protruding above the lipid bilayer are in close agreement with crystal structure data and the topological asymmetry of SecYEG allows orientation determination. From volume calculations we are able to differentiate SecYEG monomers from dimers and higher order oligomeric states. Images of SecA bound to lipid (i.e, in the absence of SecYEG) were also obtained. In this case, the heights of SecA bound to the lipid are significantly different than the heights of SecY·A suggesting distinct binding modes of SecA to lipid compared to SecA to SecYEG. Further experiments and analysis will be required to conclusively determine the oligomeric state of active SecYEG during translocation.

1138-Pos Board B30
Association/Dissociation of the Nucleotide-Binding Domains of an ATP-Binding Cassette (ABC) Exporter during the ATP Hydrolysis Cycle
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ATP-binding cassette (ABC) proteins constitute one of the largest protein families, and most are membrane transport proteins. Eukaryotic exporters in this group, including the multidrug resistance protein P-glycoprotein, have been linked to chemotherapy resistance due to their extrusion of anticancer agents from cells. Like its prokaryotic homolog MsbA, P-glycoprotein has a functional core composed of two transmembrane domains and two nucleotide-binding domains (NBDs). Substrate transport is driven by ATP hydrolysis, but the mechanism of hydrolysis is controversial. The proposed mechanisms can be broadly classified as: 1) monomer-dimer models, where alternating access depends on a large separation of the NBDs (up to 50 angstroms) during the ATP binding/hydrolysis cycle, or 2) constant-contact models, where the NBDs remain associated during the cycle. To address the ABC exporters' mechanism, we used lanthanide-based resonance energy transfer (LRET) to follow the intramolecular movements of MsbA during the ATP binding/hydrolysis cycle. Although the MsbA NBDs sample three discrete positions during the cycle, the average NBD separation decreases dramatically after the addition of ATP to the Apo (nucleotide-free) protein. ATP binding reduced the predominant NBD separation distance from 50 to 35 angstroms. ATP hydrolysis elicited by MgATP relaxed a fraction of the NBD dimer population towards the Apo state. These steady-state distance measurements are consistent with a monomer-dimer model. The correlation between rates of NBDs dissociation (determined from kinetic LRET measurements) and ATP hydrolysis are consistent with monomer-dimer models, where the NBDs dissociate during each hydrolysis cycle. This work was supported by a grant from CPRIT grant RP101073 and an American Heart Association Pre-doctoral Fellowship 11PRE7360046 to RSC.

1139-Pos Board B31
ATP Hydrolysis at One Site Drives the Dissociation of ATP-Binding Cassette Nucleotide-Binding Domains
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Typical ATP Binding Cassette (ABC) transporters are membrane proteins that couple the energy from ATP hydrolysis to transport of an extended variety of substrates, including nutrients, toxins, peptides and even ions. Basically they are formed by two transmembrane domains and two nucleotide binding domains (NBDs). Currently, there are two main models to explain the conformational changes that occur in the NBDs in response to ATP binding and hydrolysis: a switch-model that proposes NBDs association/dissociation, and a constant-contact model with the NBDs always in contact during the transport cycle. Recently, using Luminescence Resonance Energy Transfer (LRET) and